Colonization of the Grass Webworm, *Herpetogramma licarsisalis*, and its Adaptability for Laboratory Tests[1]

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Since its discovery in Hawaii in 1967 (Davis 1968), the grass webworm, *Herpetogramma licarsisalis* (Walker), has become the major insect pest of turfgrass in Hawaii. Damage is caused by the larvae feeding on the leaves, stems and crown of the plants, causing large patches of lawn to die in severe infestations. Colonization of this insect for obtaining quantities of eggs, larvae, or adults would open up avenues of laboratory and greenhouse research for such standard procedures as insecticidal screening. Therefore, studies were conducted to develop practical methods of rearing the insect for such purposes.

Simultaneously with developing a rearing technique, a detailed study of the life history of the insect was conducted. From the latter it was determined that a generation was completed every 32 days at an average temperature of 24.5° C. Average periods in days required were: preoviposition 5.2, egg incubation 5, larval period 14.3 and pupal period 7.3. During an average longevity of 13 days females deposited an average of 249 eggs (Tashiro, 1976).

Preliminary Studies. Full-grown to nearly full-grown larvae collected in turf plots at the Waimanalo Experimental Farm were held in plastic trays and fed one of several available grass leaves until pupation. The following grasses were readily consumed by larvae: California grass, *Brachiaria mutica*; Sunturf Bermuda grass, *Cynodon magennisii* Hurcombe; Pangola grass, *Digitaria decumbens*; centipedegrass, *Eremochloa ophiuroides* (Munro) Hack; kikuyugrass, *Pennisetum clandestinum* Hhchst ex Chiov.; and St. Augustine grass, *Stenotaphrum secundatum* (Walt.) Kuntz. It appeared that any grass with soft leaves would serve as larval food. The least practical of the acceptable grasses after cutting was Sunturf bermuda. It dried so rapidly after removal from the plant that it became unpalatible within 24 hours.

After pupation webworms were transferred to screen and aluminum cages for adult emergence, mating, and oviposition. Each cage was 24 cm on all sides, with a glass front and an 8.8 cm diam. hole in the rear for entry, and was fitted with a dixie cup as a closure. When adults emerged, cut Sunturf bermuda in a plastic cup containing water was introduced as an oviposition medium. From the initial larval collection 7 male and 7 female moths emerged. Seven days later a total of 335 eggs were found; 101 on Sunturf

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bermuda blades, 179 on the surface of the plastic cup, 25 on moist paper toweling and 30 on the thatch. Color varied from cream to orange and the eggs were assumed to have been deposited during the 3 preceding days.

Sunturf blades with attached eggs were transferred to 10-15 cm diam. pots of Sunturf and held in an outdoor cage. Within 7 days it was apparent that larvae had hatched and were growing well, as evidenced by consumption of grass and surface webbing. Larval recoveries of 16, 20, 12 and 12 were made from the 4 pots, prepupae were present by the 18th day and adult emergence began by the 28th day. During the next 10 days 63 male and 28 female moths emerged, and it appeared that successful mass rearing was well under way. More definite procedures were established from this preliminary rearing study.

Environment of Rearing Area. All rearing was done in the laboratory where the temperature averaged 24.5°C during the study period, and relative humidity ranged between 60 and 80%. Natural daylight was supplemented with fluorescent lighting an average of 8 hours a day, 5 or 6 days a week, but this supplemental lighting was not considered essential for successful rearing.

Adult Emergence and Oviposition. Since sex ratio was determined to be nearly 1:1 (Tashiro, 1976) all pupae of a given age were placed in an oviposition cage without sexing and pairing. Upon emergence adults were immediately supplied either 10% sucrose or 10% honey solution, dispensed with a dental roll from a plastic vial or baby food jar. An anti-fungal agent, 0.1% methyl paraban, was incorporated in the solutions to retard mold formation.

The need for more than water for oviposition and longevity was determined by placing a pair of freshly emerged moths in each cage and introducing growing Sunturf bermuda in a 25 x 53 mm plastic vial as an oviposition medium. The 4 treatments included no water, water only, 10% honey and 10% sucrose. These were dispensed from 25 x 53 mm plastic vials with dental rolls. Three pairs of moths placed in separate cages were used for each treatment. The daily egg production of each female and day of death of each moth were recorded. Results are given in Table 1.

With no water, all moths were dead within 4 days with no oviposition occurring. With water only provided, the females averaged only 41 eggs and were all dead within 6 days. Moths fed either honey or sucrose solutions were productive for 12 to 18 days and deposited means of 274 and 421 eggs, respectively. The last of females in these treatments died on the 18th day. There were no significant differences between moths fed honey or sucrose, but it was evident that either was necessary for maximum oviposition and longevity.

In laboratory rearing of Lepidoptera, various oviposition media other than food plants have been used with much success. As examples, wax paper is provided for codling moth, *Laspeyresia pomonella* (L.) (Hamilton and Hathaway 1966); paper toweling for cabbage looper, *Trichoplusia ni* (Hubner) (Henneberry and Kishaba 1966); and filter paper for pink bollworm, *Pectinophora gossypiella* (Saunders) (Martin 1966). Surfaces for grass webworm oviposition presented in addition to grass leaves were: wax paper, moist and dry blotter paper and smooth and crumpled paper toweling. None of these surfaces were accepted as a suitable oviposition substrate. Even in the absence of grass, only the wax paper received a few eggs, all of them deformed and non-viable. The only acceptable oviposition substrates were the upper surface of grass blades and the surface of plastic vials and plastic plant pots. On these surfaces eggs were deposited along the rim of the vials and near the drainage holes of the pots adjacent to areas of moisture.

When single pairs were confined, Sunturf bermuda growing in a plastic vial (25 x 53 mm) provided sufficient leaf surface for oviposition. Exchange of plants daily to every second day was found to be advisable since bermuda growing in shade etiolated rapidly. When 10 or more pairs of moths were confined, Sunturf growing as a solid sward in 5-10 cm plastic pots and maintained at 1-1.5 cm cutting height was an ideal oviposition medium.

Larval Rearing. Larval rearing was conducted by 2 different methods. In the first, well embryonated eggs were transferred to Sunturf bermuda and kikuyugrass growing in 15 cm diam. pots and maintained outdoors as short turf. Pots were fertilized heavily to obtain the most vigorous growth during the larval feeding period. After infesting, the pots were placed in an outdoor cage to protect them from other turf insects, and watered as needed. When hatching and larval feeding became evident, webbing on the grass was even more evident. This procedure was best adapted for colony maintenance rather than for rearing larvae for use. Larval maturity and cessation of feeding in preparation for pupation was always evident since the grass immediately started to recover.

The populations of larvae that 15 cm diam. pots of grass would support without loss of larvae was studied. Major loss of larvae resulted through exhaustion of food and larval migration out of the pot. Potted Sunturf received 10, 20, and 30 eggs each and potted kikuyugrass received 30, 40, and 50 eggs each. There were 4 pots in each treatment. The recovery of adults from this test are shown in Table 2. Only the Sunturf pots with 10 eggs each and kikuyu pots with 30 eggs each had any green leaves left at the height of the feeding period. All others were entirely devoid of food supply, but the 20 egg pots of Sunturf appeared to have the food supply exhausted at about the time of pupation. In most of the other combinations, larvae had migrated out of the pots and were found either on the floor of the cage or in the plastic tray in which the pots were placed. Apparently, some of the Sunturf pots with 10 eggs each were invaded by other larvae which explains why more than 10 moths per pot were recovered. Kikuyugrass, being a more rapid grower, supported larger colonies of larvae. This advantage was partially offset by the need to attach a long plastic sleeve to each pot and support it with stakes to confine the elongating stems. This rearing procedure appeared to be best adapted for obtaining adults for the breeding stock, and maintenance of the colony. The use of 6 in. pots of Sunturf bermuda infested with 20 eggs each was considered to be the best procedure for routine colony maintenance.

In the second method, plastic trays measuring 25 x 32 x 8 mm deep and containing cut kikuyugrass were each infested with about 200 mature eggs, or larvae that had hatched the previous night. Cut grass was added as needed, care being taken not to supply excessive amounts of food that would increase the problem of molding. Moisture regulation was the most critical consideration since lack of sufficient moisture caused the grass to dry beyond palatability, but excessive moisture allowed the growth of mold on the unconsumed food and grass. This situation became progressively more acute as larvae grew and the amount of frass increased. Regulation of moisture was achieved by first using solid covers while larvae were very small and the grass in the trays was releasing little moisture. As larvae grew and more unconsumed grass and frass accumulated, lids with screen were used to permit moisture escape. Some relief from excessive moisture was obtained by lining the trays with paper toweling. Finally, lids of mostly screen were used as larvae approached pupation. Also, larval populations in each tray were reduced to less than 100/tray during the final larval stadium. Placing fresh food on one side of the tray to force larvae to move onto it and thereby make it possible to discard the old food, was not very feasible, as only a portion of the larvae migrated to the new food supply.

Larval rearing in trays was best suited for obtaining larvae for uses other than colony maintenance. Rearing in trays required the daily attention of supplying food and regulating moisture, and larval mortality was greater than with the pot method. A comparison of moth emergence from rearings in potted Sunturf, potted kikuyu, and in trays of kikuyu is shown in Table 3. After pupation and before adult emergence began, each grass pot was placed in a screen cage, as was the contents of each rearing tray, including pupae, remaining grass stems, and all the frass. Adults were removed and counted daily and the cages were held until adult emergence had ceased. In pots of bermuda and kikuyu, moth emergence continued for 13 days and adult recovery was 69% and 63% from Sunturf and kikuyu, respectively. From trays with cut kikuyu, adult emergence continued for 10 days with a 40% adult recovery. The shorter survival period with the latter method was attributed to a slightly higher average temperature in the laboratory during the period when this method was tested.

Suitability of Grass Webworm for Laboratory Insecticide Tests. To determine the suitability of grass webworm larvae for use in laboratory insecticidal tests, a quantity of eggs of the same age were held in a plastic tray for hatching and molting to the second instar. Fifteen cm diam. pots of Sunturf maintained as short turf were infested with 25 young 2nd instar larvae each and held for 3 days to permit larvae to adapt to their new environment. Each pot was then treated with chlorpyrifos by sifting preweighed quantities of a 0.5% granular formulation onto the grass surface. When treated the larvae were in their 3rd stadium. Rates of application were equivalent to 0, .125, .25, .5 and 1.0 lb. AI/A. Each treatment was replicated with 4 pots. Treated pots were arranged in a randomized complete block design in an outdoor cage. The only attention given was daily watering to maintain the grass. When larvae approached maturity in the untreated pots, all pots were transferred to the laboratory and covered with aluminum foil during the late afternoon. This caused the larvae to remain on the surface

Table 1. *Effect of adult diet on fecundity and longevity of female grass webworm moths.*

Liquid provided moths	Avg. no. eggs deposited/female on days following emergence and pairing 3-4	5-6	7-8	9-10	11-12	13-18	Mean total eggs/female[a]
None	0[b]						0[d]
Water only	41	0					41[d]
Honey sol.[c]	97	77	54	43	3	0	274[e]
Sucrose sol.[c]	87	124	109	71	16	14	421[e]

[a] Means followed by same letter not significantly different at 5% level according to Duncan's multiple range test.

[b] Death of last female.

[c] 10% solutions + 0.1% methyl paraben.

Table 2. *Recovery of grass webworm moths from eggs placed on Sunturf bermuda and kikuyugrass growing in 15-cm pots.*[a]

Grass	No. eggs introduced /pot	Avg. no. moths emerging/pot[b]	% recovery
Sunturf	10	10.3	100
	20	15.0	75
	30	14.0	47
Kikuyu	30	19.5	65
	40	19.3	48
	50	27.7	55

[a] Pots maintained in outdoor cage.
[b] 4 single-pot replications/treatment.

TABLE 3. *Comparison of grass webworm adult emergence from Sunturf bermuda growing in 15-cm pots, kikuyugrass growing in 15-cm pots and harvested kikuyugrass in plastic trays.*

	% of total moth emergence		
Days of emergence	Potted Sunturf	Potted kikuyu	Harvested kikuyu
i	4.8	3.0	1.5
2	17.5	4.2	5.1
3	11.4	12.4	24.5
4	6.7	25.3	25.5
5	10.8	23.4	24.0
6	13.9	11.3	8.7
7	17.5	7.2	7.7
8	5.4	5.3	2.6
9	8.4	3.0	0
10	.6	2.6	.5
11	2.4	1.5	
12	0	.3	
13	.6	.3	
Total Moths Emerged:	116	265	196
♂:♀ ratio	1:1.1	1:1.03	1:1.18
No. eggs released:	240	420	490
% emergence as moths:	69.2	63.1	40.0

TABLE 4. *Dose-morality relationship of chlorpyrifos to grass webworm larvae investing potted Sunturf bermuda.*

Lbs. AI/A	*Mean recovery/pot*[a] Larvae	Adults	Mean total /pot[b]	% reduction
1.0	0	0	0 a	100
.5	3.3	1.0	4.3 ab	70.9
.25	6.3	2.0	8.3 bc	43.9
.125	9.5	2.5	12.0 cd	18.9
0	12.5	2.3	14.8 d	—

[a] 4 single 15-cm pot replications/treatment.

[b] Means followed by the same letter not significantly different at 5% level; Duncan's multiple range test.

the next morning when they were counted and removed. This method of taking larval counts is an adaptation of the procedure used by Mitchell and Murdoch (1974) for insect counts in turf field plots. After counting, the foil was reapplied, and counts were made again at the end of the day. Finally, the pots were held for adult emergence by covering each pot with a sheet of plastic and securing with a rubber band. The results of this study are shown in Table 4. Average recovery from the untreated checks was 14.8/pot. Recovery from all the pots of all the treatments yielded 79% as larvae and 21% as adults. Total population reductions of 18.9, 43.9, 70.9 and 100% of untreated checks was obtained with the .125, .25, .5 and 1.0 lb./A rates, respectively. This test demonstrated the suitability of grass webworm larvae for use in laboratory insecticidal tests.

ABSTRACT

The grass webworm, *Herpetogramma licarsisalis* (Walker), was readily colonized in the laboratory and outdoor screened cage. Moths required either a honey or sucrose solution for maximum longevity and fecundity. Oviposition occurred mainly on the upper surface of Sunturf bermuda grass leaf blades, but also on plastic vials and plant pots adjacent to areas of moisture. Larval rearing was accomplished on potted Sunturf bermuda, potted kikuyugrass and on cut kikuyugrass in plastic trays. Pupae were held in 24x24x24 cm cubical screen and glass cages where adults emerged and mating and oviposition occurred. Minimum generation time was 32 days at 24.5°C. Application of 0.5 G formulation of chlorpyrifos to the surface of pottted Sunturf bermuda grass infested with third instar larvae demonstrated that this insect is suitable for laboratory insecticidal screening tests.

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